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MOUSE ANTI HUMAN CD49d MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB1383

LOT NUMBER: 20101026

QUANTITY: 100 µg

CONCENTRATION: 1.0 mg/mL

SPECIFICITY: Recognizes the 80kDa alpha chain of VLA-4. Reacts with monocytes, T cells, B cells, thymocytes and Langerhans cells. Can be used in basic studies of VLA-4 mediated adhesion and its interaction with the VCAM-1 structure. HP2/1 inhibits cell binding to soluble VCAM-1.

IMMUNOGEN: JM leukemia line

ISOTYPE: IgG1

CLONE NAME: HP2/1

APPLICATIONS: Flow cytometry: 1µg/5 X 10^5 cells
Immunohistology on frozen tissue sections
Immunoprecipitation
Optimal working dilutions must be determined by end user.

SPECIES CROSS-REACTIVITY: Rat, pig, rhesus monkey

FORMAT: Purified immunoglobulin - Ig fraction

PRESENTATION: Liquid in Phosphate Buffered Saline, pH 7.4 with 0.1% Sodium Azide.

STORAGE: Store at -20°C in undiluted aliquots. May be stored at 2-8°C for short term use. Avoid repeated freeze-thaw cycles.

REFERENCES:

1. Sanchez-Madrid, F. et al. (1986). *Eur. Journal Immunology* **16:** 1343-1349.
2 Weller et al. (1991). *P.N.A.S. USA* **88:** 7430.
3. Mattila, P. et al. (1992). *Int. J. Cancer* **52:** 918-923.
4. Leukocyte Typing Workshop V, p. 1646-1648.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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4-99/MAB1383/KG/TRD

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MOUSE ANTI-HUMAN INTEGRIN α 4 MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB1955

LOT NUMBER: 18080112

QUANTITY: 100 µL

SPECIFICITY: α4 integrin. The α4β1 integrin receptor recognizes an RGD-independent alternative adhesion site in the CS-1 region of fibronectin.

ISOTYPE: IgG_3

CLONE NAME: P4C2

APPLICATIONS: Immunofluorescence and immunoprecipitation.

Inhibits attachment of hematopoeitic cells and T-lymphocytes but not fibroblasts to fibronectin; typical titer is >1:1,000
Aggregation of Jurkat cells to 1:1600 dilution

FORMAT: Liquid ascites containing sodium azide as a preservative.

STORAGE/HANDLING: Store at -20°C in undiluted aliquots for up to 12 months. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Wayner, E.A., *et al., J. Cell Biol.* **109**:1321 (1989).
2. Wayner, E.A., *et al., J. Cell Biol.* **121**:1141 (1993).
3. Rahman, S. et al. (1998). *Biochem. J.* **335:** 247-257.

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Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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MOUSE ANTI-HUMAN INTEGRIN α4 MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB1954

LOT NUMBER: 20020923

QUANTITY: 100 µL

SPECIFICITY: Human integrin α4. The α4β1 integrin receptor recognizes an RGD-independent alternative adhesion site in the CS-1 region of fibronectin.

ISOTYPE: IgG_{2b}

CLONE NAME: P4G9

APPLICATIONS: Immunocytochemistry on Jurkat cells at 1:1,600.
Inhibits attachment of hematopoeitic cells and fibroblasts to fibronectin (note: sodium azide should be removed prior to attachement assay).
Optimal working dilutions must be determined by the end user.

FORMAT: Ascites.

STORAGE/HANDLING: Store at – 20 °C in undiluted aliquots for up to 12 months. Avoid repeated freeze/thaw cycles.

REFERENCES:

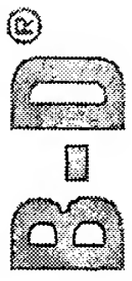
1. Wayner, E.A., *et al., J. Cell Biol.* **109**:1321 (1989).
2. Wayner, E.A., *et al., J. Cell Biol.* **121**:1141 (1993).
3. *Exp. Hematology* **24**:158-168 (1996).
4. *J. Biol. Chemistry* **274(43):** 30906-30913 (1996).

For research use only; not for use as a diagnostic.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

Monoclonal Antibodies Detecting Human Antigens

CD49d (Anti–VLA-α4)



Pure	Catalog No. 340976	200 µg	(0.01% azide, 1 mg/mL)
PE*	Catalog No. 340296	50 Tests	

DESCRIPTION

Specificity

CD49d (Anti–VLA-α4) recognizes the 150-kilodalton (kd) α chain of very-late antigen (VLA)-4, a member of the integrin family of cell adhesion molecules.[1-3] VLA-4, like other integrins, is a noncovalently associated heterodimeric glycoprotein composed of α and β subunits and is involved in cell–cell and cell–extracellular matrix interactions.[1-4] The β chain of the VLA-4 complex is the CD29 antigen, a 130-kd glycoprotein.[2] The CD29 antigen, also known as the β1 subunit, is common to the VLA family of integrins.[1,2,4] When acting as a matrix receptor, the CD49d antigen binds to CS-1, an alternatively spliced domain of fibronectin.[1,2,5,6] When functioning as a cell receptor, the CD49d antigen binds to the vascular cell-adhesion molecule-1 (VCAM-1).[1,4,5] The interaction between the CD49d antigen and VCAM-1 is known to play an important role in stabilizing the adhesion of lymphocytes to endothelial cells[4,7] and in mediating B-lymphocyte precursor/bone marrow stromal cell adhesion.[5] The CD49d antigen, when associated with the β7 integrin, forms a lymphocyte homing receptor for Peyer's patch, binding to the mucosal vascular addressin MAdCAM-1.[8] The CD49d antigen is also involved in CD3-dependent $CD4^+$ T-lymphocyte activation via its interaction with fibronectin.[6,9]

Antigen Distribution

The CD49d antigen is primarily expressed on T and B lymphocytes and weakly expressed on monocytes.[1,10]

Functional Characteristics

CD49d (Anti–VLA-α4) can block or enhance fibronectin-stimulated T-lymphocyte proliferation.[9,11] It immunoprecipitates three proteins of 150 kd, 85 kd, and 75 kd under both reducing and nonreducing conditions from HPB-ALL cells, B lymphoblasts, peripheral blood lymphocytes, and IL-2–dependent cell lines.[10]

Clone

CD49d (Anti–VLA-α4), clone L25, is derived from the fusion of mouse Sp2/0 cells with popliteal lymph node cells from a BALB/c mouse immunized with a $CD8^+$ T-cell line.[10,12]

Ig Chain Composition

CD49d (Anti–VLA-α4) is composed of mouse IgG_{2b} heavy chains and kappa light chains.

RESEARCH APPLICATIONS

Studies of:

- leucocyte integrins[2]
- cell–cell and cell–extracellular matrix adhesion[1,2]
- B-lymphocyte precursor/bone marrow stromal cell adhesion[5]
- T-lymphocyte cytokine production[13–18]
- signal transduction and T-cell activation[6,9,11,15,17,19]
- hematogenous tumor metastasis and expression of integrin α4 on melanoma, Burkitt's lymphoma, and kidney carcinoma cells[20,21]
- immunoprecipitation of integrin α4[10]
- interactions between integrin α4 and VCAM-1[22]

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* US Patent No. 4,520,110; European Patent No. 76,695; Canadian Patent No. 1,179,942

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DIRECT IMMUNOFLUORESCENCE

Product/Amount for Staining

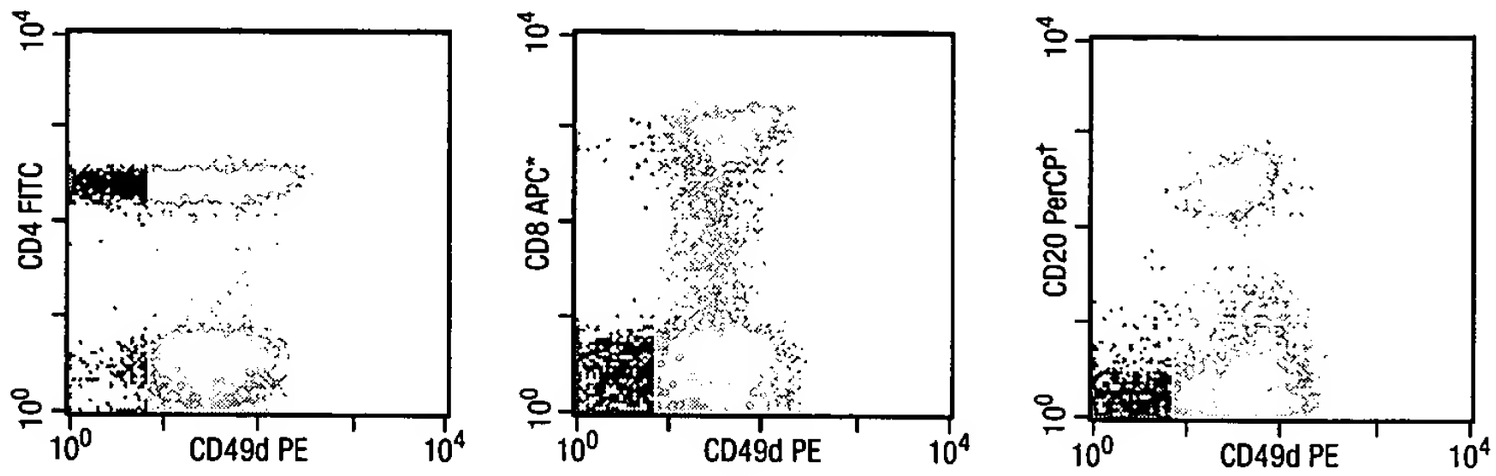
CD49d (Anti–VLA-α4) PE
Cat. No. 340296
20 µL/test

Method for Direct Immunofluorescence

Refer to our website (www.bdfacs.com) or your local BD representative for the lyse/wash method for direct immunofluorescence.

Representative Data

Performed on whole blood. Laser excitation is at 488 nm.



Analyzed with a FACS™ Brand Flow Cytometer

HANDLING AND STORAGE

The monoclonal antibody is supplied as 200 µg purified immunoglobulin in 0.2 mL (1.0 mg/mL) of phosphate-buffered saline (PBS) containing 0.01% sodium azide without gelatin. The PE conjugate is supplied as 1.6 µg in 1.0 mL (1.6 µg/mL) of PBS containing gelatin and 0.1% sodium azide. Vials should be stored at 2° to 8°C. Conjugated forms should not be frozen and should be protected from prolonged exposure to light. Each reagent is stable for the period shown on the bottle label when stored as directed.

WARRANTY

The products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, that extend beyond the description on the label of the product. Becton Dickinson's sole liability is limited to either replacement of the products or refund of the purchase price. Becton Dickinson is not liable for property damage, personal injury, or economic loss caused by the product.

CHARACTERIZATION

To ensure consistently high-quality reagents, each lot of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data are included in this data sheet.

WARNING

Reagents contain sodium azide. Sodium azide is harmful if swallowed. Keep out of reach of children. Keep away from food, drink, and animal feedingstuff. Wear suitable protective clothing. If swallowed, seek medical advice immediately and show this container or label. Contact with acids liberates very toxic gas. Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

REFERENCES

1. Springer TA. Adhesion receptors of the immune system. *Nature.* 1990;346:425-434.
2. Albelda SM, Buck CA. Integrins and other cell adhesion molecules. *FASEB J.* 1990;4:2868-2880.
3. Modderman PW. New clusters: CD29/CDw49, CD47 CD51, CD55, and CD61. In: Knapp W, Dörken B, Gilks WR, et al, eds. *Leucocyte Typing IV: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1989:1017-1019.
4. Elices MJ, Osborn L, Takada Y, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct form the VLA-4/fibronectin binding site. *Cell.* 1990;60:577-584.
5. Dittel BN, McCarthy JB, Wayner EA, LeBien TW. Regulation of human B-cell precursor adhesion to bone marrow stromal cells by cytokines that exert opposing effects on the expression of vascular cell adhesion molecule-1 (VCAM-1). *Blood.* 1993;81(9):2272-2282.
6. Nojima Y, Humphries MJ, Mould AP, et al. VLA-4 mediates CD3-dependent CD4⁺ T cell activation via the CS1 alternately spliced domain of fibronectin. *J Exp Med.* 1990;172:1185-1192.
7. Lin Y, Kirby JA, Browell DA, et al. Renal allograft rejection: Expression and function of VCAM-1 on tubular epithelial cells. *Clin Exp Immunol.* 1993;92:145-151.

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* US Patent No. 4,520,110; European Patent No. 76,695; Canadian Patent No. 1,179,942

† US Patent No. 4,876,190

8. Berlin C, Berg EL, Briskin MJ, et al. Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell.* 1993;74:185.

9. Shimizu Y, Van Seventer GA, Horgan KJ, Shaw S. Costimulation of proliferative responses of resting CD4$^+$ T cells by the interaction of VLA-4 and VLA-5 with fibronectin or VLA-6 with laminin. *J Immunol.* 1990;145:59-67.

10. Clayberger C, Krensky AM, McIntyre BW, et al. Identification and characterization of two novel lymphocyte function-associated antigens, L24 and L25. *J Immunol.* 1987;138:1510-1514.

11. Davis LS, Oppenheimer-Marks N, Bednarczyk JL, McIntyre BW, Lipsky PE. Fibronectin promotes proliferation of naive and memory T cells by signaling through both the VLA-4 and VLA-5 integrin molecules. *J Immunol.* 1990;145:785-793.

12. Hemler ME, Kassner P, Bodorova J. CD49d cluster report. In: Schlossman SF, Boumsell L, Gilks W, et al, eds. *Leucocyte Typing V: White Cell Differentiation Antigens.* New York, NY: Oxford University Press; 1995:1617-1618.

13. Rotteveel FTM, Kokkelink I, van Lier RA, et al. Clonal analysis of functionally distinct human CD4$^+$ T cell subsets. *J Exp Med.* 1988;168:1659-1673.

14. Waldrop SL, Davis KA, Maino VC, Picker LJ. Normal human CD4$^+$ memory T cells display broad heterogeneity in their activation threshold for cytokine synthesis. *J Immunol.* 1998;161:5284-5295.

15. Pitcher CJ, Quittner C, Peterson DM, et al. HIV-1–specific CD4$^+$ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. *Nat Med.* 1999;5:518-525.

16. Maino VC, Picker LJ. Identification of functional subsets by flow cytometry: intracellular detection of cytokine expression. *Cytometry.* 1998;34:207-215.

17. Suni MA, Picker LJ, Maino VC. Detection of antigen-specific T cell cytokine expression in whole blood by flow cytometry. *J Immunol Methods.* 1998;212:89-98.

18. Maino VC. Rapid assessment of antigen induced cytokine expression in memory T cells by flow cytometry. *Vet Immunol Immunopathol.* 1998;63:199-207.

19. Udagawa T, McIntyre BW. A VLA-4 α-chain specific monoclonal antibody enhances CD3-induced IL-2/IL-2 receptor-dependent T-cell proliferation. *Lymphokine Cytokine Res.* 1992;11:193-199.

20. Taichman DB, Cybulsky MI, Djaffar I, et al. Tumor cell surface alpha 4 beta 1 integrin mediates adhesion to vascular endothelium: demonstration of an interaction with the N-terminal domains of INCAM-110/VCAM-1. *Cell Regul.* 1991;2:347-355.

21. Rice GE, Bevilacqua MP. An inducible endothelial cell-surface glycoprotein mediates melanoma adhesion. *Science.* 1989;246:1303-1306.

22. Shimizu Y, Newman W, Gopal TV, et al. Four molecular pathways of T cell adhesion to endothelial cells: roles of LFA-1, VCAM-1, and ELAM-1 and changes in pathway hierarchy under different activation conditions. *J Cell Biol.* 1991;113:1203-1212.